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IMMUNOFLUORESCENCE IN THE FIELD OF LUPUS ERYTHEMATOSUS

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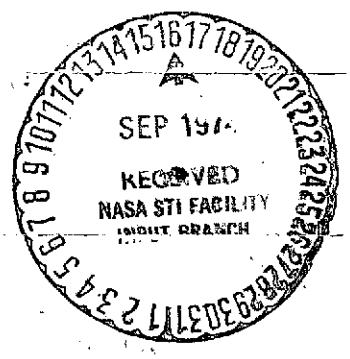

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16. Abstract

The search for antinucleus antibodies by means of immunofluorescence is important in the course of acute disseminated Lupus Erythematosus both diagnostically, because the antinucleus antibody and other autoantibodies allow its serologic analysis, and also pathogenetically, because the multiplicity of autoantibodies shows a deep disturbance of immunoregulation. In addition, the localization of depositis of immunoglobulin in target organs (skin and kidney) seems to be related to the deposition of immune complexes containing antinucleus antibodies. These expressions of the disease have a direct pathogenic role in its determination. Acute disseminated Lupus Erythematosus and chronic Lupus appear thus to be not only diseases with autoimmunization, but also deriving from autoimmunization.

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IMMUNOFLUORESCENCE IN THE FIELD OF LUPUS ERYTHEMATOSUS

J. Thivolet
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Immunofluorescence is widely used in the study of Lupus Erythematosus: /187*

-- above all, in the search for diagnostically useful autoantibodies,
-- in the histoimmunological study of tissue lesions.

These two aspects will be analyzed separately.

1) Search for Autoantibodies Using Immunofluorescence

In 1948 the discovery by Hargraves of the LE cell brought a new element, fundamental for the diagnosis, which previously could only be done clinically and histopathologically. The nucleophagocytosis taking place in the patients' clotted blood has been easily interpreted by the presence of antibodies in the serum of patients affected with acute disseminated Lupus Erythematosus (ADLE) (?).

In fact, the phenomenon is reproducible when the patient's serum acts on polynuclears from normal subjects.

The "Haserick factor" contained in these sera is an immunoglobulin, and more precisely an antinuclear autoantibody. These antinuclear antibodies attach themselves on the nuclei and form with them AG-Ac, complement-fixing complexes. The complement attracts, by chemotaxis, live normal polynuclears, which phagocytize these nuclei causing three alterations: swelling, modification of structure, and of staining properties.

The search for LE cells, directly or indirectly (Haserick

*Numbers in the margin indicate pagination in the foreign text.

test) is semeiologically important, but presents some difficulties: the need for an accurate technique, false positive reaction (tart cells), low sensitivity, especially in patients undergoing treatment.

Since the LE phenomenon takes place for the presence of antinuclear antibodies, other immunological techniques have successfully been used to detect these antibodies. There are many such techniques, but the search using immunofluorescence is still the most popular, because of its technical simplicity and its sensitivity. The general principle is well known, and nuclei of different tissues and of different animals can be used as antigens, since these autoantibodies have no organ -- or species -- specificity.

Some examples follow.

The antinuclear antibodies are really a complex group of autoantibodies in which the following have been differentiated: anti-denatured DNA antibodies, pure or mixed; anti-deoxyribonucleoprotein antibodies; antibodies against certain ill-defined nuclear constituents, which confer to immunofluorescence certain non-homogeneous aspects.

The semeiological value of antinuclear antibodies is subject to two factors:

aA) <u>The antibody titer</u>: high titers, of 1024 or more in the immunofluorescence, are specific for ADLE; lower titers may be found in other affections (viral, such as infectious mononucleosis; hepatic; cutaneous, such as bullous pemphigoid, and above all, PCR, scleroderma, etc.).

B) The type of antibodies: the anti-DNA antibodies are the most characteristic of ADLE, especially eruptive, and before treatment; but this phenomenon is observed inonly 51% of the patients; the anti-nucleoproteinantibodies (of homogeneous appearance in immunofluorescence) are present in 80% of the patients, but are found also in other affections. Among the antibodies which give a nonhomogeneous appearance, the antinucleus reticulated antibodies are found in17% of the ADLE, but also in scleroderma.

In practice, the following general rules may be stated:

1) The anti-DNA antibodies, or the association of these antibodies with the anti-deoxyribonucleoprotein (anti-DNP) of high titer are characteristic of ADLE (acute disseminated Lupus Erythematosus); /188

2) The absence of every antinucleus antibody excludes the diagnosis of ADLE.

3) The anti-DNP antibodies with antibodies of the reticulated type with high titer are indicative of ADLE, but may be observed in other diseases, especially PCR, scleroderma, chronic active hepatitis.

4) Antinucleus antibodies of various types at low titers may be the expression of various circumstances:

a) of a latent lupus diathesis;
b) of an "epiphenomenal" autoimmune reaction in case of extensive tissue destruction;
c) of a degradation of the immunoregulatory system in older subjects.

5) In ADLE, the titers of the antinucleus antibodies fluctuate in the patient in relation to the course and treatment of the disease; but this parallelism has very broad margins.

6) In chronic Lupus Erythematosus, the antinucleus antibodies are found in highly variable percentages, according to statistics, but always in low titers (10-30%). This semeiological observation may disclose the lupous diathesis and allow joining chronic and acute Lupus in a single pathologic entity.

Further serological anomalies: their semeiological value is varible, because they are not always accompanied by the presence of antinucleus antibodies.

Let us examine some of them:

A) Various autoantibodies have been demonstrated, in particular: (1) antiglobulin antibodies (anti-Ig type rheumatoid factor IgM); (2) cryoglobulins which may interpret the genesis of certain symptoms (Raynaud's syndrome; necrotic purpurus); (3) autoantibodies against various cell constituents, and finally, anticollagen autoantibodies.

B) Perhaps more important is the finding of antiplatelet, antileukocyte, and antierythrocyte autoantibodies which allow the assessment of thrombopenia, leukopenia, and hemolytic anemias of ADLE. In addition, circulatory anticoagulants have been reported which are similar to prothrombin anticomplex autoantibodies.

C) The false biological reactions of syphilis are not rare in ADLE; they involve a positive reaction in hemolysis and in flocculations, using cardiolipinic antigens, and correspond to a true autoantibody phenomenon, since the cardiolipinic antigen is a tissue antigen. Finally, nonspecific reactions in the

immunofluorescence test, due to antinucleus antibodies, have recently been reported.

This group of autoantibodies is accompanied by hyperglobulinemia, often associated with a lowering of the serum complement, especially evident in the course of "poussés" in the evolution of ADLE, and in the case of severe nephropathy.

In conclusion, the search for antinucleus antibodies by means of immunofluorescence is important in the course of ADLE, not only diagnostically, because the antinucleus antibody and the other autoantibodies allow its serologic analysis, which is fundamental for the differentiation of the disease, but also pathogenetically, because the multiplicity of the autoantibodies shows a deep disturbance of immunoregulation.

2) Immunopathologic Data

The discovery by Vasquez and Diton in 1957 of globulin deposits in the kidneys and in the spleen during the course of ADLE opened the way to numerous studies which, using mainly immunofluorescence techniques, have made it possible to specify immunopathologic data and their relationship with the autoantibodies on the one hand, and with the physiopathology of the lesions on the other. Two target organs have been the main objects of these studies, kidneys and skin; we shall emphasize the results obtained with skin biopsies.

Direct immunofluorescence methods were used in sections of biopsies, frozen and sliced at -22°C. Antisera specific against the various globulins IgG, A, M, D; C, fibrin, etc. make it possible to visualize the deposits of these proteins. The immunohistochemical study may be carried out also following labeling of certain enzymes, especially peroxidase, which also allows ultrastructural study with the electron microscope.

The observations made on skin biopsies have allowed, following the studies of Burnhamm and Cormane, observation of the deposit of immunoglobulins localized at the dermal-epidermal junction, /189 in a more or less continuous band, of variable aspect in thick fluorescent granules, mixed with more grayish granules, sometimes almost threadlike.

The C (complement?) is often present, and also fibrin and albumin can be observed. Deposits of perivesicular globulins are also observed around the dermal vessels. Finally, more seldom antinucleus antibodies are observed on the nucleus of the epidermal and dermal cells. It has further been observed that these deposits, and especially the dermo-epidermal junction band, could be found occasionally also in the healthy skin, and more often in the areas exposed to light. It will be remembered that similar deposits have been attested in the kidney glomeruli; and at the level of the vessels of various organs, especially the spleen.

The frequency of cutaneous deposits differs markedly between healthy, normal skin and acute disseminated Lupus Erythematosus and chronic Lupus Erythematosus.

In the diseased skin the globulin band is found in practically all cases of ADLE and CLE. The absence of these deposits excludes this diagnosis; vice versa, the study of various dermatoses has permitted the observation that the band may be present in some of them (linear bullous pemphigus, rosacea; lichen ruber planus, porphyria cutanea).

In the healthy skin, the globulin band is found only in ADLE, in percentage of about 60%.

The mode of formation and the pathogenetic role of the deposits of tissue immunoglobulin in LE have elicited much

research in which the leading idea has been supplied by the study of experimental nephropathies due to immunocomplexes.

Immunization caused by repeated antigen injection leads to formation of an antigen-antibody complex circulating in an excess of antigen, and to formation of glomerulopathies with deposits of globulin and of complement on the basal glomerular membrane.

Activated complement exerts chemotaxis on the polynuclears which destroy the glomerular basis through liberation of lysosomal enzymes and of vasoactive amines (bradiquinine, histamine), which control the penetration of the complex at the level of the basal [membrane]. This mechanism has been confirmed in the course of several human diseases causing glomerulonephritis and for which the suggested active factors are viral antigens (virus coxackie virus, lymphocytic choriomeningitis), protein antigens (seric disease), nucleus antigens, and finally, anti-DN antigens in ADLE nephropathies.

Concerning the skin deposits, we have used a microtechnique of acid elution of antibodies from the globulin deposits. Analysis of the eluates has shown the presence of antinucleus antibodies. These studies, as well as our previous ones on kidney deposits, show that the antinucleus antibodies have a direct pathogenic role and that ADLE may be considered a disease caused by autoimmune complexes.

In addition, we have isolated another kind of antibody, an antibasal cutaneous membrane antibody. The interpretation of this unexpected discovery is still open (one could think of an alteration of the basal membrane due to deposit of immunoglobulin, leading to a secondary autoimmunization against antigens coming from the membrane].

Some electron microscope studies have permitted the observation in the normal skin of ADLE of some electron-dense granules under the basal membrane and in the vascular walls; these dense deposits, which have been observed also in the glomerular basal [membrane], might be considered a substrate of antigen-antibody complexes.

In conclusion, the localization of deposits of immunoglobulin in target organs such as the skin and kidney seems to be related to the deposition of immune complexes containing antinucleus antibodies. These are not only the expression of the disease, but have a direct pathogenic role in its determination. ADLE and CLE appear thus to be not only diseases with autoimmunization, but deriving from autoimmunization.

V.A. Puccinelli

Many thanks to Prof. Thivolet for his brilliant report. He also has exerted himself in a reading with which he is not familiar.

I shall not emphasize the outstanding aspects of his communication, because we know most of them from the literature; there are, however, some original aspects that I wish to emphasize, and which we appreciate; for example, the possibility of differentiating, through quantitative analysis, the various forms of Lupus Erythematosus and, above all, the discovery of a variety of anti-basal cutaneous membrane antibody. This is very interesting, not only because it is original, but because it shows that the basal membrane is capable of acting as an antigen. /190

We shall now conclude the clinical dermatology part with some observations by Giannetti on immunofluorescence in certain special dermatoses. These are isolated findings, showing however

how the application of immunofluorescence techniques are bound to expand and to be the source of useful data, especially in the clinical field. After this, Leigheb will report to us on the most recent findings on the FTA-ABS, a reaction well known to all of you in the current serologic practice of syphilis.